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DIRECTIONS

FOR

LABORATORY WORK

IN

PHYSIOLOGICAL CHEMISTRY.

FOR THE USE OF STUDENTS IN THE UNIVERSITY AND
BELLEVUE HOSPITAL MEDICAL COLLEGE.

BY
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FIRST THOUSAND.

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No. 1938

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PREFACE.

As the title indicates, this little book is intended especially as a guide to the students in the course in Physiological Chemistry as given in the University and Bellevue Hospital Medical College. It makes no pretence of being more than this. On this account the subjects outlined are limited in number, while the individual experiments are described with considerable detail.

It is my desire to express here my indebtedness to Dr. John A. Mandel, Professor of Physiological Chemistry in this Institution, at whose suggestion this manual was prepared.

H. C. J.

October, 1902.

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LABORATORY NOTES
FOR
PHYSIOLOGICAL CHEMISTRY.

CARBOHYDRATES.

Tests for constituent elements.

(*a*) *Carbon.*—1. Heat cautiously a small particle of substance on a platinum foil. The piece will char, due to the separation of the carbon in the substance. Further heating renders the carbon capable of combining with the oxygen of the air, with the result that the former passes off as CO_2. In the case of carbohydrates, when the combustion is complete, no residue is obtained. A substance containing oxygen in sufficient quantities to form CO_2 with all the carbon present will not carbonize.

2. Mix thoroughly some of the dried substance with powdered CuO and place the mixture in the bottom of a dry test-tube. Upon warming, the carbon of the substance is oxidized by the oxygen of the CuO and escapes as CO_2. This CO_2 may be detected by holding a glass rod moistened with lead acetate at the mouth of the test-tube.

(*b*) *Hydrogen.*—1. In the latter experiment, moisture will have collected on the cold part of the test-tube. The hydrogen of the substance, in the presence of heat, has combined with the oxygen supplied by the CuO, forming H_2O.

MONOSACCHARIDES, $C_6H_{12}O_6$.

Dextrose. Levulose. Galactose.

DEXTROSE = grape-sugar = glucose.

Tests: (*a*) *Moore's.*—To 5 c.c. of the dextrose solution add an equal volume of NaOH, and heat. The mixture becomes yellow and finally brown, due to the formation of caramel (?). This test lacks delicacy and reliability in examining urine.

(*b*) *Trommer's.*—To 5 c.c. of the dextrose solution add an equal volume of NaOH. Then add, drop by drop, a dilute solution of $CuSO_4$ (so dilute that the green color is just visible) until a trace of permanent precipitate (?) remains. The solution should be deep blue in color. Warm the upper part of the solution and note result. Write all the equations for the reactions taking place in this experiment.

(*c*) *Fehling's.*—Heat 5 c.c. of Fehling's solution (?) just to boiling and add a few drops of the dextrose solution. Continue boiling until the solution commences to respond as with Trommer's test. Compare the reactions of this test with those of the previous ones.

(*d*) *Barfoed's.*—To 5 c.c. of Barfoed's reagent (?) add a few drops of the dextrose solution and boil. Note the result and write equations.

(*e*) *Nylander's.*—To 5 c.c. of the dextrose solution add 10 drops of Nylander's reagent (?) and boil. The solution gradually turns yellow and finally black, bismuth (?) being precipitated.

(*f*) *Silver Nitrate.*—To 5 c.c. of $AgNO_3$ solution in a *clean* test-tube add dilute NH_4OH, drop by drop, until the precipi-

tate, which is at first formed, dissolves. Then add a few drops of the dextrose solution and warm on the water-bath. Note the formation of a metallic mirror on the side of the test-tube. Explain chemical changes.

(*g*) *Phenylhydrazin.*—In a test-tube make a mixture of 5 drops phenylhydrazin, 10 drops glacial acetic acid, and 1 c.c. of a saturated solution of NaCl; then add 5 c.c. of the dextrose solution and boil for a few minutes. Yellow phenylglucosazone crystals will appear on cooling. These must be examined under the microscope. This is the most distinctively characteristic and conclusive of all tests for dextrose. The crystalline form is the important point. Other substances form osazones, but these latter differ in crystalline form, solubilities, and melting-points. Write the chemical equations for the formation of osazones.

(*h*) *Fermentation.*—In a test-tube shake 20 c.c. of the dextrose solution with a small piece of compressed yeast. Place the mixture in a saccharometer and allow it to stand in a thermostat at 40° C. A gas (?) collects at the top of the tube and the amount is in a direct ratio to the amount of dextrose in the solution. What is the character of the chemical changes which have taken place?

DISACCHARIDES, $C_{12}H_{22}O_{11}$.

Maltose. Lactose. Saccharose.

SACCHAROSE = cane sugar = sucrose.

Tests: (*a*) Fehling's; Moore's; Nylander's; Fermentation; Barfoed's; using in each case 5 c.c. of the saccharose solution. Compare the results with those obtained with dextrose.

(*b*) To 10 c.c. of the saccharose solution add 1 c.c. concentrated HCl and boil several minutes. Allow to cool and then make *alkaline* with NaOH. Use this solution in making Fehling's, Nylander's, and Barfoed's tests. Write the equations for the chemical changes which have taken place and determine the character of the carbohydrate formed.

POLYSACCHARIDES, $(C_6H_{10}O_5)_n$.

Starchs. Dextrins. Glycogen. Celluloses.

STARCH.

(*a*) Examine under the microscope and sketch the following starch granules: potato, corn, wheat, and rice.

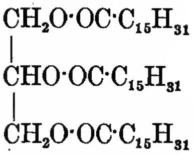
(*b*) Place some starch in a test-tube half full of water and shake thoroughly until the starch is finely divided. Heat water to boiling in another test-tube and add to this enough of the cold starch mixture to make a translucent solution (about 5 per cent.). What takes place upon pouring the suspended starch into the hot water? The solution of starch thus obtained is called a paste, and in making experiments with this substance such a *paste* must always be prepared.

(*c*) To 5 c.c. of starch paste add a drop of iodine solution. Warm gradually and then allow to cool. Note changes.

(*d*) Boil 10 c.c. of starch paste with 1 c.c. of concentrated HCl for 5 minutes. Observe the change which the solution undergoes. Neutralize part of this *cold* solution with NaOH, and apply Fehling's and Barfoed's tests. (If no reduction appears, continue the boiling of the original acid solution for some minutes longer and repeat the neutralization and tests.) Determine the character of the sugar causing this reduction.

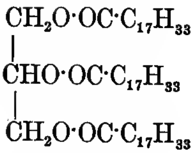
FATS.

Tripalmitin.



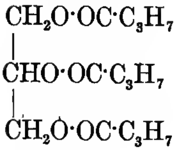
$$\begin{array}{l} CH_2O\cdot OC\cdot C_{15}H_{31} \\ | \\ CHO\cdot OC\cdot C_{15}H_{31} \\ | \\ CH_2O\cdot OC\cdot C_{15}H_{31} \end{array}$$

Trioleïn.



$$\begin{array}{l} CH_2O\cdot OC\cdot C_{17}H_{33} \\ | \\ CHO\cdot OC\cdot C_{17}H_{33} \\ | \\ CH_2O\cdot OC\cdot C_{17}H_{33} \end{array}$$

Tributyrin.



$$\begin{array}{l} CH_2O\cdot OC\cdot C_3H_7 \\ | \\ CHO\cdot OC\cdot C_3H_7 \\ | \\ CH_2O\cdot OC\cdot C_3H_7 \end{array}$$

Try the following tests, making use of olive oil (?):

(*a*) Test its solubility in water; ether; chloroform; alcohol.

(*b*) Test its reaction. What is the normal reaction of a fat? What is *rancid* butter?

(*c*) In a test-tube warm a few drops with potassium bisulphate. Notice odor. What is the reaction taking place?

(*d*) Let a drop of an ether solution of a fat fall upon paper.

(*e*) Shake some olive oil with water and allow to stand.

(*f*) *Saponification.*

1. Place a piece of bayberry wax (tripalmitin) in an evaporating-dish and add water until it is half full. Then add about 10 c.c. NaOH and boil until the substance is dissolved, adding water from time to time as the solution evaporates, so that the *original volume is constantly maintained.* Write the reaction which is taking place. After complete solution, add carefully dilute H_2SO_4 until the precipitation is complete and then cool. A greenish crust of free fatty acid will form on the surface of the liquid, so that the solution may be poured off. (Keep this solution.) Break the fatty acid crust into small pieces and wash with tap water; finally dry between filter paper. Dissolve part of the fatty acid in hot 95 per

cent alcohol, filter through dry funnel and allow the filtrate to cool slowly.

Palmitic acid separates out in snowy-white crystalline form. Examine under the microscope.

2. Place 50 c.c. of alcoholic potash (?) in a flask containing 25 grams of lard. Warm the mixture upon the water-bath until a drop let fall into water is perfectly soluble. Then pour the solution into an evaporating-dish containing 100 c.c. water and evaporate until the alcohol has been driven off. While still hot precipitate with H_2SO_4 and allow to cool. Write the chemical equations for the various steps in the procedure. The fatty acid again forms a crust which may be easily removed from the liquid. Unite this solution with the similar one in Experiment 1 and, after neutralization with Na_2CO_3, evaporate down to about 5 c.c. What substances are present in this solution? Retain this.

Perform the following reactions with the *fatty-acid* crust:

(*a*) Test solubility in ether; chloroform; water; alcohol.

(*b*) Warm in a dry test-tube with potassium bisulphate.

(*c*) Shake up the remainder of the fatty acid with 15 c.c. water and add NaOH, drop by drop, until the acid is all dissolved. Write the reaction. What is formed?

Use this soap solution for the following reactions:

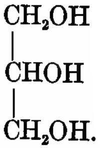
(*a*) Add 5 drops to a saturated solution of NaCl.

(*b*) Take 10 c.c. of tap water and add the soap solution, drop by drop with shaking, until the precipitate fails to redissolve. The foam which also previously formed and then disappeared, now is permanent. Explain these conditions.

(*c*) Shake some olive oil with water and add a few drops of the soap solution. What is an emulsion? Examine under the microscope. Albumins and gums form similar emulsions with fats. This emulsion of fats by means of a soap solution is important on account of the probability that this

reaction plays a decided part in the absorption of fat from the intestine.

GLYCERINE,



$$\begin{array}{l}\mathrm{CH_2OH}\\ \quad|\\ \mathrm{CHOH}\\ \quad|\\ \mathrm{CH_2OH.}\end{array}$$

Make use of the solution obtained from the two saponifications for the following tests:

(*a*) Note the taste and try its solubility in alcohol; ether; water.

(*b*) Add some dry potassium bisulphate and warm. Compare this with the same reaction under fats. Write equation.

(*c*) Test with Fehling's solution.

(*d*) Make a faint precipitate of $Cu(OH)_2$, and to this add the remainder of the glycerine solution. What happens?

PROTEIDS.

Divided into *simple proteids*, *compound proteids*, and *albuminoids*.

REACTIONS GENERAL TO ALL PROTEIDS.

Proteids contain *carbon*, *hydrogen*, *oxygen*, *nitrogen*, and *sulphur*, sometimes *phosphorus* and *iron*.

Test the substance for *carbon* and *hydrogen*.

(*a*) Test the white of egg (coagulated) for *nitrogen* as follows: Make an intimate mixture of this substance with soda lime and introduce it into a dry test-tube. Warm gently. Hold a piece of moistened red litmus paper or a glass rod moistened with HCl at the mouth of the test-tube. What is the reaction which takes place?

(*b*) Warm carefully a small quantity of the dry substance in a *dry* test-tube with a small piece of metallic sodium. When the sodium ignites remove the tube from the flame and after the ignition has ceased plunge the warm end into a conical glass quarter full of water. Filter the solution, and to the filtrate add a few drops of ferric chloride and ferrous sulphate. Upon acidifying with HCl a blue precipitate of ferro-ferricyanide (?) is formed. Write out the chemical reactions which take place in this manipulation.

Sulphur is present in proteids in two forms, viz.: Loosely combined, i.e., combined with H; example: $-SH$. Firmly combined, i.e., combined with O; example: $-SO_2$.

Test for *loosely combined* sulphur as follows:

Place about 5 c.c. of dilute NaOH in a test-tube with a small quantity of the substance, and add two drops of lead acetate. Heat the mixture for a few minutes and note changes. In what form is this kind of sulphur split off by NaOH? What causes the blackening?

Test for *oxidized* sulphur as follows:

Mix some of the substance with double its quantity of the fusion mixture ($Na_2CO_3 + KNO_3$). Place the whole in a small porcelain crucible and warm *cautiously* until the mixture becomes colorless. (If the fusion begins to sputter, remove the flame.) The residue (fused mass) should be nearly white. It is then dissolved in 10 c.c. dilute HCl, filtered, and the filtrate precipitated warm with $BaCl_2$. What is this white precipitate? Write the reaction. How is this sulphur split off?

Phosphorus.—Fuse some casein (?) with the fusion mixture as in the previous experiment. Dissolve the fused mass in 10 c.c. of water acidified with HNO_3. Filter and add

5 c.c. of ammonium molybdate solution. Warm some minutes at about 80° C. What is the yellow precipitate?

Iron.—Incinerate a small quantity of hæmoglobin (? in a porcelain crucible. The ash should be red. Dissolve out the ash with 10 c.c. of dilute HCl. Filter. Test this solution for Fe with potassium sulphocyanide or poatssium ferrocyanide.

COLOR REACTIONS.

For the following tests make use of the white-of-egg solution as a typical example of proteid:

(*a*) *Xanthoproteic Test.*—To 5 c.c. of albumin solution add 10 drops HNO_3. Note the white precipitate (?). Boil until the precipitate is dissolved and the solution becomes light yellow. *Cool,* and add an *excess* of NH_4OH. The color changes to orange. Other substances may give a yellow solution with HNO_3, but the solution does not respond orange upon neutralization with NH_4OH. What are the chemical changes of this reaction?

(*b*) *Millon's Test.*—Add a few c.c. of Millon's reagent (?) to 5 c.c. of the albumin solution. The precipitate (?) formed turns red slowly upon heating. This reaction is suitable for solids or liquids where the presence of salts in the solution is not excessive. What atomic complex in the proteid molecule causes this reaction? What simple substance also gives this reaction?

(*c*) *Biuret Test.* Suited for testing *solutions* only.—Place 5 c.c. of the albumin solution in a test-tube and add an equal volume of NaOH. Then add, drop by drop, a $CuSO_4$ solution so dilute that the color is hardly visible. The color obtained will vary from blue violet to reddish violet, according to the kind of proteid in solution. If too much $CuSO_4$ is added the solution becomes green. A specific atomic complex in the proteid molecule is also accountable for this reaction. What simple substance will respond to this test?

(*d*) *Adamkiewicz Test.*—Boil 2 c.c. of the albumin solution with 5 c.c. of glacial acetic acid until a complete solution is obtained. Cool, and cautiously run down the side of the test-tube 2 c.c. concentrated H_2SO_4. A violet color develops at the junction of the two liquids.

PRECIPITATION REACTIONS.

(*a*) *Heller's Test.*—If 5 c.c. of HNO_3 is placed in a test-tube and a few c.c. of the albumin solution allowed to flow gently down the side of the test-tube and stratify itself on top of the HNO_3, a white ring of precipitated albumin will form where the two liquids meet. HCl and H_2SO_4 will also give the same reaction.

(*b*) *Acetic acid and potassium ferrocyanide.*—Make 5 c.c. of the albumin solution acid with acetic acid and add, drop by drop, a *dilute* solution of potassium ferrocyanide. Note result.

(*c*) Acidify 25 c.c. of the albumin solution with acetic acid.

1. To 5 c.c. of this solution add 2 drops of tannic acid solution.
2. To 5 c.c. of this solution add 2 drops of picric acid solution.
3. To 5 c.c. of this solution add 3 volumes of 95 per cent. alcohol.
4. To 5 c.c. of this solution add $MgSO_4$ (salt) to saturation.

5. To 5 c.c. of this solution add $(NH_4)_2\ SO_4$ (salt) to saturation.

Note results and see if the precipitation is complete in each case.

(*d*) Acidify 10 c.c. of the albumin solution with HCl.

1. To 5 c.c. of this solution add 2 drops of phosphotungstic acid.
2. To 5 c.c. of this solution add 2 drops of potassium-mercuric-iodide.

(*e*) To successive portions of 5 c.c. of the albumin solution add a few drops of $CuSO_4$; neutral and basic lead acetate; $HgCl_2$; trichloracetic acid.

Make careful notes of the results of the above reactions, and where possible write equations.

SIMPLE PROTEIDS.

ALBUMINS.

(*a*) Coagulation. Heat 5 c.c. of the albumin solution and then add one or two drops of acetic acid. The albumin separates out in an insoluble, flocky (coagulated) form. Why is the addition of the acetic acid necessary? Try to dissolve the coagulum in some of the ordinary proteid solvents. Make 5 c.c. of the albumin solution faintly alkaline, and heat. Note differences.

(*b*) To 10 c.c. of the albumin solution add $(NH_4)_2SO_4$ to saturation. Note result and compare with experiment (*c*) 5, p. 11. Filter off the precipitate and test the filtrate with the Biuret test.

(*c*) To 10 c.c. of the albumin solution add $MgSO_4$ to saturation. Compare this result with that of experiment (*c*) 4,

p. 11. Now add 2 drops of acetic acid. What is the precipitate?

(*d*) Test the albumin solution for loosely combined sulphur.

(*e*) Warm some dried albumin in a *dry* test-tube to about 100° C. Then try to dissolve it in water. Why does not the albumin coagulate by the heat?

GLOBULINS.

(*a*) Try two proteid color reactions and three precipitation reactions; see if the solution will coagulate.

(*b*) Globulins are insoluble in water but dissolve in weak salt solutions. They may be precipitated from such solutions by the removal of the salts by dialysis or by decreasing the percentage of salt in the solution by dilution. Pour the globulin solution, drop by drop, into a beaker full of water.

(*c*) Start dialysis experiment (see demonstration).

(*d*) Saturate 10 c.c. of the globulin solution with $MgSO_4$. Filter off precipitate (?) and test filtrate for proteid. Compare this reaction with the similar one for albumin.

(*e*) Cool 10 c.c. of the globulin solution under the tap and let CO_2 gas bubble through it. Note result.

(*f*) Take 10 c.c. of blood-serum and saturate it with $MgSO_4$. What is this precipitate? Filter, and to the filtrate add two drops of acetic acid. What is this second precipitate? Test both precipitates for proteid.

ACID AND ALKALI ALBUMIN.

To 15 c.c. of the albumin solution add 10 drops of HCl and warm for 15 min. at 40° C. Cool.

(*a*) *Exactly* neutralize one half of this solution with very dilute NaOH. What is this precipitate? Shake up the precipitate and divide the solution into two parts. To the first

add a drop of NaOH and heat. To the other heat and then add a drop of NaOH. Explain the differences in results.

(*b*) Try three characteristic proteid tests on the remaining solution.

To 15 c.c. of the albumin solution add 10 drops of NaOH and warm for 15 minutes at 40° C. Cool.

(*a*) Try the effect of heating this solution.

(*b*) Neutralize the solution.

(*c*) Try three characteristic proteid tests.

COMPOUND PROTEIDS.

MUCINS (GLYCOPROTEIDS).

Add 30 c.c. of saliva to 100 c.c. of 95 per cent alcohol.

Filter off the precipitated mucin and make the following tests:

(*a*) Try some color-reactions for proteids.

(*b*) Dissolve some of the precipitate in weak NaOH and then add acetic acid drop by drop. Note results.

(*c*) Boil the remaining precipitate for 10 minutes in a small flask with 50 c.c. of ·5 per cent HCl. Cool, neutralize with NaOH and test for reducing body with Fehling's solution. Explain the result.

ALBUMINOIDS.

COLLAGEN (GELATIN).

Collagen occurs as the chief constituent of connective tissue (cartilage) and also as the organic matrix of bone (ossein). By boiling with water or weak acids, it is easily converted into gelatin, water being added to the molecule. The process is one of hydration. Collagen is therefore the anhydride of gelatin. Collagen is very resistant to reagents, tending to dissolve it unchanged, so we will study its hydrated product gelatin. The bone from which the salts have been removed is cut up into small pieces and placed in an evaporating-dish half full of acidulated water. The mixture is kept boiling until the pieces of collagen, or in this case ossein, are dissolved. (Add water from time to time as the solution concentrates.) Upon cooling, the solution will gelatinize unless it contains less than 1 per cent of gelatin.

Make the following tests, dissolving the jelly in hot water as it is needed:

(*a*) Biuret; (*b*) Millon's; (*c*) Acetic acid and potassium ferrocyanide; (*d*) Tannic acid; (*e*) HCl or H_2SO_4; (*f*) Saturation with $(NH_4)_2SO_4$; (*g*) Loosely combined sulphur. Note and compare the results carefully with those obtained with albumin.

KERATINS.

The chief constituent of hair, nails, hoof, horns, feathers, etc.

Their main characteristic is insolubility, and large percentage of loosely combined sulphur.

Use horn shavings for the following reactions:

(*a*) Loosely combined sulphur; (*b*) Millon's; (*c*) Xanthoproteic; (*d*) Try its solubility in water, acids, and alkalies.

MUSCULAR TISSUE.

Reaction.—Test the reaction of living and dead muscle to litmus and congo red paper. Explain the differences in the results obtained.

PROTEIDS.

Place 25 grms. of hashed fresh muscle in a beaker with 75 c.c. of water and allow to stand, with frequent stirring for one hour. Strain off the muscle through some cloth (keep the residue) and filter the solution. Test the filtrate for proteid and determine the nature of such proteid.

MYOSIN.

Digest the above meat residue with 100 c.c. of 15 per cent NH_4Cl solution for twenty-four hours in a covered beaker. Then filter and use the filtrate for the following reactions:

(*a*) Pour a little of the solution, drop by drop, into a beaker filled with water. Compare this reaction with a similar one under globulins.

(*b*) Heat a few c.c. of the filtrate. Filter and test filtrate for Ca.

(*c*) Saturate 10 c.c. of the filtrate with $MgSO_4$. Filter and test the filtrate for proteid.

(*d*) Try Millon's, Biuret, and Xanthoproteic tests.

NITROGENOUS EXTRACTIVES.

Creatin.—Extract 250 grms. chopped beef with 250 c.c. water for half an hour over the water-bath at 50° C. Strain through muslin as dry as possible and make a second extraction in the same way, with an equal amount of water (save residue). Unite the two extractions, add 2 or 3 drops of acetic acid and evaporate to small volume. Remove the coagulated proteids by filtration, and to the filtrate add *carefully* basic lead acetate, *avoiding any excess*; the precipitate consists of phosphates, chlorides, sulphate, etc. Allow the precipitate to settle and then filter. Warm the filtrate and pass

H_2S through it to remove the excess of lead. Filter hot. The filtrate should be water-clear. Evaporate the filtrate on a water-bath to a thin syrup. Upon standing several days in a cool place crystals of creatin will deposit. Remove the crystals, placing them in a small flask with 10 c.c. dilute H_2SO_4, and heat half an hour on the water-bath, keeping the volume constant. While still warm add $BaCO_3$, in substance, to neutralization. Filter and evaporate the filtrate to 10 c.c. The creatin has been changed to creatinin. Write the equation. Perform the following tests with the solution:

(*a*) Place 2 drops of the solution upon a watch-glass and add to it a few drops of an alcoholic solution of $ZnCl_2$; allow to stand for several days and then examine the crystals under the microscope.

(*b*) *Weyl's Reaction.*—To 2 c.c. of the creatinin solution add three drops of a dilute solution, freshly prepared, of sodium nitroprusside. Then add, drop by drop, dilute NaOH. A ruby-red color is produced which quickly changes to yellow. If the solution is now acidified with acetic acid and boiled, a green color is obtained, and upon continued boiling a precipitate of prussian blue settles out.

(*c*) Treat some of the solution with a dilute solution of picric acid, and make it faintly alkaline with NaOH. The solution immediately becomes a deep red. Acetone responds to this test, but gives merely a yellowish red.

GLYCOGEN $(C_6H_{10}O_5)_n$.

For extraction and preparation of glycogen from liver, muscle or scallop, see demonstration.

Perform the following reactions with the solution thus obtained:

(*a*) Notice the color of the solution. Add 2 drops of the iodine solution to 5 c.c. of the glycogen solution. Warm gently and allow to cool. Note changes.

(*b*) Test 5 c.c. of the solution with Fehling's solution.

(*c*) To 5 c.c. of the solution add three volumes of 95 per cent alcohol.

(*d*) To 5 c.c. of the solution add 5 drops of concentrated H_2SO_4 and boil for a few minutes. Neutralize with NaOH and test with Fehling's solution. What is this reducing substance? How could you prove it?

(*f*) To 5 c.c. of the solution add a few drops of filtered saliva, and warm at 40° C. for a few minutes. Notice changes in the solution. Determine the character of the final reducing body formed.

BONE.

Made up of organic matrix (ossein) and inorganic salts. *Ossein* has been studied under Collagen.

MINERAL CONSTITUENTS.

Incinerate 10 grms. of bone under the hood. Boil out the grayish residue with 25 c.c. of dilute HNO_3, filter, and use the filtrate for the following tests:

(*a*) *Phosphoric acid.*—To 10 c.c. of the solution add 2 c.c. of the molybdic solution and warm in the water-bath at 75°C. until a canary-colored precipitate settles out. What is this? Filter off on a small filter, wash once with very dilute HNO_3, and then dissolve the precipitate upon the filter by adding dilute NH_4OH drop by drop. To the filtrate add a few c.c.

of magnesium mixture (?). What is this precipitate? Write all the equations.

(b) *Chlorides.*—To a few c.c. of the solution add a few drops of $AgNO_3$. What results?

(c) *Calcium.*—Make 10 c.c. of the solution alkaline with NH_4OH. What is the precipitate? Filter, and to the filtrate add ammonium oxalate. The white precipitate of calcium oxalate is proof of the presence of calcium combined other than with phosphoric acid.

(d) *Magnesium.*—To 10 c.c. of the solution add NH_4OH in excess and then acetic acid until an acid reaction is obtained. The precipitate (?) obtained upon the addition of NH_4OH dissolves for the most part in the acetic acid. The residue (?) is filtered off, dissolved in dilute HCl and tested for iron by potassium ferrocyanide. To the filtrate add ammonium oxalate and filter off the precipitate (?). Make this filtrate alkaline, and add sodium phosphate. A precipitate shows the presence of magnesium?

(e) *Carbonates.*—Did you notice an effervescence when you added HNO_3 after incineration?

(f) *Sulphates.*—Make some of the solution alkaline with NH_4OH and then dissolve the precipitate with HCl again. Add a few drops of $BaCl_2$. What is this precipitate? Write the equations for all these reactions.

SALIVARY DIGESTION.

CHEMICAL CHARACTERISTICS.

Collect about 50 c.c. of filtered saliva.

(*a*) Test its reaction with litmus paper. To what is this due?

(*b*) To a few c.c. of saliva add acetic acid, drop by drop, until a precipitate forms. (See under Mucin.) Filter off the mucin and test the filtrate for proteid. What is the result?

(*c*) Allow a couple of drops of saliva to drop on a piece of filter paper. Then add a drop of ferric chloride to the paper where it is moist. Note color. Now add a drop of mercuric chloride. What is the result? For what substance are these tests?

(*d*) Prepare a test-tube full of starch paste and allow it to cool to 40° C. Dilute the saliva five times and regulate the water-bath for 40° C.

AMYLOLYSIS.

Take 15 c.c. of the starch paste; add to it 1 c.c. of the diluted saliva and place the test-tube in the water-bath. Watch the digestion until the turbidity disappears and the solution becomes transparent. Then pour out into a clean tube 1 or 2 c.c. of the digesting solution and add a drop of iodine solution. What color is given? Replace the digestion in the bath. As the digestion proceeds remove successive portions (2 c.c.) and test with the iodine solution. What successive variations in color do you obtain? Finally the iodine fails to give a color to the solution. This is called the achromic point; why? Test the remainder of the digestion with Fehling's solution. What is the character of the sugar present? What causes these changes as indicated by the iodine reaction? What are the successive hydrolytic products formed in the digestion?

(*e*) Make up the following mixtures in separate test-tubes and keep them in the water-bath at 40° C, using in each case 5 c.c. of the diluted saliva.

1. Saliva + 10 c.c. starch paste.
2. Saliva boiled and then cooled + 10 c.c. starch paste.
3. Saliva neutralized with HCl + 10 c.c. starch paste.
4. Saliva made acid with HCl + 10 c.c. starch paste.
5. Saliva made alkaline with NaOH + 10 c.c. starch paste.

In all these test-tubes note carefully the changes which are taking place from time to time, testing the rapidity of digestion by means of the iodine reaction. Determine the varying lengths of time necessary for the achromic point to be reached.

After some time it will be noticed that in experiments 4 and 5 the starch pastes have not changed; change the reaction in each to that corresponding to the normal saliva and again allow them to digest. What result?

Make deductions from these test-tube experiments.

GASTRIC DIGESTION.

Tests for detecting *hydrochloric acid* and *lactic acid* separately and together.

Test each of the following solutions with all the indicators mentioned below, and *tabulate* the results whether positive or negative.

(*a*) 0.3 per cent HCl; (*b*) 0.05 per cent HCl; (*c*) 0.8 per cent lactic acid; (*d*) a mixture containing equal volumes of

"*a*" and "*e*"; (*e*) a mixture containing equal volumes of "*b*" and a 2 per cent albumose solution.

Indicators.

1. *Dimethylamidoazobenzol*, $N(CH_3)_2-C_6H_4-N=N-C_6H_5$. Add one or two drops *directly* to the solution to be tested. Free mineral acid is indicated by a carmine-red color.

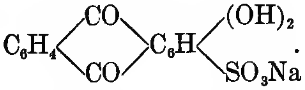
2. *TropæolinOO*, $NH(C_6H_5)-C_6H_4-N=N-C_6H_4-SO_3Na$. Add one or two drops *directly* to the solution to be tested. Free acid is indicated by a red or reddish violet color.

3. *Congo red.*—Use congo red paper, prepared by dipping filter paper into the alkaline indicator solution and drying. Free acid is indicated by the blue color.

4. *Günzburg's Reagent* (phloroglucin, 2 grms.; vanillin, 1 grm.; alcohol, 100 c.c.). Evaporate 2 drops of the solution to be tested with 1 drop of the indicator, in a porcelain dish, *carefully*, over a water-bath. Upon dryness the presence of free HCl is indicated by the development of a rose-red color.

5. *Boas' Reagent* (resorcin, 5 grms.; saccharose, 3 grms.; alcohol, 100 c.c.). Mix 3 to 5 drops of solution to be tested with the same amount of indicator and evaporate cautiously in a porcelain dish. Free acid is shown by a rose or vermillion color.

6. *Alizarin S*,



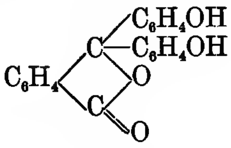
$$C_6H_4{<}\begin{matrix}CO\\CO\end{matrix}{>}C_6H{<}\begin{matrix}(OH)_2\\SO_3Na\end{matrix}.$$

Add 2 or 3 drops of the indicator directly to the solution.

Indicator reacts yellow in acid and acid salt solutions, but red to violet in alkaline.

This indicator is especially suited for the determination of the acidity of the urine by titration.

7. *Phenolphthaleïn,*



Add 2 drops of the indicator directly to the solution.

The indicator is colorless in neutral and acid reactions, but becomes red in the presence of alkalies. It shows the presence of free, combined, mineral and organic acids, and acid salts of all kinds. It therefore gives the total acidity. It may indicate acid reaction when dimethylamidoazobenzol shows alkaline. Explain this.

Lactic Acid in the Presence of HCl.

Uffelman's Reagent (1% carbolic acid solution faintly colored with Fe_2Cl_6). With this solution test solutions *a*, *c*, and *d*. Note carefully color changes and make deductions.

Make a very dilute solution of Fe_2Cl_6 in which the yellow color is hardly visible. Test solutions *a*, *c*, and *d* with this. Such a reagent is much more sensitive than Uffelman's. Use the dilute ferric chloride solution in testing the following: H_2NaPO_4; alcohol; saccharose and glucose. Make deductions as to the value of the test applied *directly* to gastric contents.

In order that all chances for error may be avoided, lactic acid may be easily separated from disturbing conditions by shaking the stomach contents or gastric juice with ether in which the acid is soluble.

Such an ether extract may be evaporated carefully on the water-bath and the residue taken up with water.

As this ether extraction cannot contain any of the above substances, the presence of which in the stomach contents

might interfere with a correct diagnosis, a positive test for lactic acid in this case is decisive evidence of its presence.

PEPSIN AND PEPSINOGEN.

1. A glycerin extract of a pig's gastric mucosa contains *pepsinogen.*

2. A 0.2% HCl extract of a pig's gastric mucosa contains *pepsin HCl.*

3. A watery extract of a pig's gastric mucosa contains *pepsin.*

Make use of these solutions numbered 1, 2, and 3 respectively, and in each test-tube add a piece of fibrin, keeping all at 40° C. in the water-bath.

(*a*) Fibrin + 5 c.c. of 0.2% HCl.
(*b*) “ + 5 c.c. of solution 3.
(*c*) “ + one drop of solution 1.
(*d*) “ + 5 c.c. of solution 2.
(*e*) “ + one drop of solution 1 + 5 c.c. of 0.2% HCl.
(*f*) “ + 5 c.c. of solution 2 (the latter having previously been heated to boiling and again cooled).
(*g*) Fibrin + one drop of solution 1 (the latter having previously been heated to boiling with 5 c.c. water and again cooled) + 5 c.c. of 0.2% HCl.
(*h*) Fibrin + 5 c.c. of solution 3 + 5 c.c. of 0.8% lactic acid.
(*k*) “ + “ “ “ 3 + “ “ 1% oxalic acid.
(*m*) “ + “ “ “ 3 + 3 drops concentrated HCl.
(*n*) “ + “ “ “ 3 + 5 “ NaOH.
(*p*) “ + “ “ “ 3 + 3 “ of bile.

Note *carefully,* in each case, the relative rapidity with which the flock of fibrin is disintegrated.

PEPTIC PROTEOLYSIS.

Make use of the solution which has been digesting at 40° C. for a week. Warm the solution in a beaker to boiling and note whether a precipitate forms. Whether this is so or not, neutralize carefully and exactly with very dilute Na_2CO_3. Filter off precipitate (?), if any, and while the filtrate is evaporating over the free flame in a porcelain dish test the precipitate as to its nature. What does the filtrate contain? When the solution is condensed to about 50–75 c.c. saturate while still hot with ammonium sulphate in substance. A yellow sticky mass will settle out, which, upon stirring with a glass rod, can be collected and stuck to the sides of the vessel so that the solution may be poured off from it into a beaker and the latter cooled under the tap. The sticky residue (proteoses) is to be dissolved in a *small* quantity of water and the resulting solution saturated with NaCl (rock salt). What proteose is precipitated? Filter and to the clear filtrate add two drops of acetic acid. Again filter off the precipitate (?). Upon saturation of this filtrate with $(NH_4)_2SO_4$ the presence of still a third proteose is shown.

The first precipitate obtained by saturation with NaCl is *Heteroproteose.*

The second precipitate upon the addition of acetic acid is *Protoproteose,* and the third precipitate is *Deuteroproteose.* The filtrate after first saturation with ammonium sulphate contains *peptone.*

This designation of the various precipitates does not indicate a sharp separation of these bodies by this simple method. The major part of these various fractions is, however, made up of the bodies indicated.

Test the three proteose precipitates with Biuret, Heller's, heat and acetic acid, and acetic acid and potassium ferro-

cyanide reactions. Make a schema of the solubility and reactions of these bodies. Prove the following facts concerning the peptone solution: Peptones are not coagulable by heat, nor do they respond to Heller's test. Millon's, Xanthoproteic, Biuret, and Tannic acid reactions yield positive results. Trichloracetic acid reaction is negative, as is also the acetic acid and potassium ferrocyanide.

RENNIN.

Make use of the rennin solution given and prepare four test-tube mixtures as follows, keeping them at 40° C. for fifteen minutes:

(*a*) 5 c.c. milk + 1 c.c. of rennin solution.

(*b*) " " + " " " " + 2 c.c. ammonium oxalate.

(*c*) 5 c.c. milk + 2 c.c. of rennin solution + 10 drops 0.3% HCl.

(*d*) 5 c.c. milk + 2 c.c. of rennin solution + 10 drops 0.5% Na_2CO_3.

Note results carefully. (*b*) has not clotted. Heat it to boiling to kill the enzyme. Cool and add a few drops of calcium chloride solution. What is this precipitate which settles out of the solution? What are the effects of acids and alkalies on the milk alone?

PANCREATIC DIGESTION.

Pancreatic digestion is the sum of three specific processes, *Proteolysis*, *Steatolysis*, and *Amylolysis*.

TRYPTIC PROTEOLYSIS.

Use pancreatic extract and prepare test-tube digestions in the water-bath at 40° C., as under gastric digestion.

1. Fibrin + 5 c.c. of pancreatic extract.
2. " + 5 c.c. " " " + 2 c.c. 0.5% Na_2CO_3.

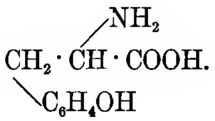
Compare the manner of action of the trypsin upon the fibrin to that of the pepsin.

3. Fibrin + 5 c.c. of 0.5% Na_2CO_3.
4. " + 5 c.c. " " " " + 5 c.c. pancreatic extract which has been previously boiled and cooled.

A pancreatic digestion has been prepared.

Take 150 c.c. of the mixture and evaporate on the water-bath to 75 c.c. Now perform the same method for the separation of proteoses and peptones as in gastric digestion. Compare carefully the results obtained with those in the case of gastric digestion. The peptone solution must now be evaporated on the water-bath until crystallization begins to occur. Then allow to cool and examine under the microscope the crusts formed. Sketch the tyrosin. Filter the solution and allow it to evaporate still further. A second crop of crystals will be formed, among which look for those of leucin. What are the differences between peptic and tryptic proteolysis?

TYROSIN.



$$\begin{array}{l} \qquad\quad NH_2 \\ CH_2 \cdot CH \cdot COOH. \\ \quad C_6H_4OH \end{array}$$

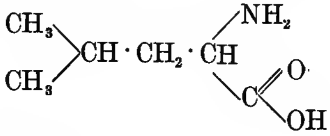
Use the powder given.

1. Try to dissolve some in cold, then warm water. Then add about 5 c.c. of Millon's reagent to the test-tube and warm. Why is this result positive?

2. *Piria's Test.*—Place a speck of the tyrosin powder upon a small watch-glass with two drops of concentrated H_2SO_4 and warm for half an hour on the water-bath. Then transfer it with about 15 c.c. water to a test-tube and neutralize with $BaCO_3$ in substance. Filter and add a few drops of weak

ferric chloride (neutral). A positive test is evidenced by the formation of a violet color.

Leucin.



Make use of powder given.

1. Place a little in a clean dry test-tube and warm carefully. The leucin sublimes, without melting, on the cold parts of the tube. If the powder is heated too high or suddenly, the substance is decomposed and the odor of amylamine (?) is given off.

2. *Scherer's Test.*—Heat a little leucin with two drops of concentrated HNO_3 upon a platinum foil over a free flame until a colorless residue is obtained. Then add two drops of NaOH and evaporate carefully in the flame. The mass becomes dark red in color and rolls around on the foil like an oil-drop.

Steatolysis.

1. To 5 c.c. of the pancreatic juice add two drops of litmus solution and a few drops of *neutral* olive-oil. Put test-tube in water-bath at 40° C.

2. Instead of olive-oil, make the same experiment using ethyl butyrate.

3. Repeat experiment 1, with the exception that the extract has previously been boiled. Compare and note these results carefully.

Amylolysis.

To 10 c.c. of starch paste add 5 c.c. of pancreatic extract, and place in the water-bath at 40° C. Test from time to time with the iodine solution as you did under salivary digestion. Do you get an achromic point? Finally test for a reducing sugar. What is the character of this?

BILE.

Note the color, consistency, and reaction of the samples presented.

Notice the color differences between the ox and dog bile.

Use the diluted bile for the following reactions:

1. Add acetic acid drop by drop to 5 c.c. of the bile. Is this precipitate mucin? How could you prove it? Filter and test the filtrate for proteid. What is the most suitable test?

2. To 5 c.c. of olive-oil add 10 c.c. of water and shake thoroughly. Allow it to stand in the rack.

3. To 5 c.c. of olive-oil add 10 c.c. of diluted bile. Allow this to stand. Compare the permanency of the emulsion.

4. To 10 c.c. of a gastric digestion mixture add diluted bile, drop by drop. What is this precipitate and its significance?

CONJUGATE BILE ACIDS.

These are present as the sodium salts of taurocholic and glycocholic acids.

Pettenkofer's Test.—Place 5 c.c. of concentrated H_2SO_4 in a clean dry test-tube. In another test-tube place 5 c.c. of diluted bile to which has been added a few drops of a 2% cane-sugar solution or a solution of furfurol (?) 1 : 1000. Pour the diluted bile carefully down the sides of the tube containing the H_2SO_4 so that the two fluids do not mix. (Method of stratification.) Notice the coloration at the line of contact of the two solutions. Shake the tube *slightly*, allowing a little more of the bile to come in contact with the H_2SO_4. The temperature must never rise above 70° C. To avoid this cool the tube under the tap. Upon careful mixing and cooling as described above, the whole solution finally becomes a cherry-red to reddish purple. Such a solution shows a definite and characteristic spectrum which distin-

guishes it from other substances, giving the same reaction, such as phenol, petroleum, fusel-oils, pyrocatechin, and cholesterin.

BILE PIGMENTS.

Bilirubin and Biliverdin.—Carnivora have larger quantities of bilirubin in their bile, while that of Herbivora contains biliverdin in predominant amounts.

Gmelin's Test.—Stratify 5 c.c. of yellow HNO_3 and 5 c.c. of diluted bile as explained in the previous experiment. Notice the play of colors at the junction of the two liquids —green, then blue, violet, red, and yellow. To what are these colors due, and what specific substances do they indicate?

Smith's Test.—Stratify 5 c.c. of diluted bile and 3 c.c. of tincture of iodine. Notice the bright green ring.

Huppert's Test.—To 10 c.c. of bile add an equal volume of milk of lime and shake thoroughly. Filter off and after washing once with water remove the precipitate (?) to a small beaker with 25 c.c. of alcohol acidulated with a few drops of H_2SO_4. Warm the beaker in a water-bath until the alcohol begins to assume an emerald-green color. What is the chemistry of this reaction?

Hammarsten's Test.—Hammarsten's reagent consists of 1 vol. HNO_3 + 19 vol. HCl. To 5 c.c. of this reagent add five drops of diluted bile. A green color immediately develops. Upon the further addition of the reagent in small quantities to the mixture, the same play of colors may be obtained as in Gmelin's test.

ANALYSIS OF A BILIARY CALCULUS.

Place some of the pulverized calculus in a clean dry test-tube with 15 c.c. ether. Shake thoroughly. Filter off the ether through a dry filter and funnel and allow it to evaporate in the air in a porcelain dish. What is the residue?

Salkowski's Test for Cholesterin.—Dissolve a small quantity of the cholesterin in a few c.c. of chloroform and add an equal volume of concentrated H_2SO_4. The acid solution takes on a greenish fluorescence and the chloroform becomes red.

Remove all the residue of the calculus remaining in the test-tube to the filter-paper used above by means of a 10% HCl solution. Then wash the precipitate upon the paper once with water and dry the paper in the funnel.

When dry pass through the filter over and over about 10 c.c. of chloroform until it assumes a yellowish color, due to its solvent action on the bilirubin. Finally allow a few c.c. of fresh chloroform to flow through the funnel, and uniting this with the original extract, let it evaporate in the air.

Use this for the following tests:

1. Gmelin's.
2. Shake a part of the chloroform extract with a dilute solution of Na_2CO_3. Notice changes in the alkaline aqueous solution.
3. Expose the aqueous solution of bilirubin to the air. What changes take place here?

To the residue of calculus still on the paper pass through over and over about 10 c.c. of ether. What does the ether extract? Evaporate off the ether by gently blowing on it. Notice the color of the residue and compare it to the bilirubin residue.

BLOOD.

GENERAL REACTIONS.

Test its reaction with litmus-paper previously moistened with a solution of concentrated NaCl. To what is this reac-

tion due? Specific gravity—see Hammarsten, p. 157, Hammerschlag's method.

Examine a drop under the microcsope.

1. To 5 c.c. of blood add 10 c.c. of water. Notice changes in the solution and examine under the microscope. What is laky blood?

2. To 5 c.c. of blood add 10 c.c. 0.8% NaCl. Examine also. What is meant by a solution *isotonic* with blood?

3. Add a few drops of bile, chloroform, and ether to successive portions of 5 c.c. of blood.

4. To 5 c.c. of blood add a few c.c. of hydrogen peroxide. To what is the frothing due? Then add a few drops of tincture of guaiacum.

Blood Serum.

1. Heat half a test-tube full of serum to boiling with the addition of a drop or two of acetic acid. Filter and test filtrate and precipitate for proteid (Millon's and Biuret reactions). Retain the filtrate.

2. Stratify 5 c.c. of serum with 2 c.c. of trichloracetic acid solution.

3. Stratify 5 c.c. of serum with 2 c.c. of Spiegler's reagent(?).

4. Acidify 5 c.c. of serum with acetic acid and add potassium ferrocyanide.

5. Test a few c.c. of the proteid-free filtrate from Experiment 1 for sugar.

6. Allow a drop of the filtrate (Experiment 1) to evaporate on a watch-glass over the water-bath. Examine under microscope. NaCl?

7. Test a few c.c. of the filtrate for chlorides.

8. To a few c.c. of oxalated plasma (?) add a few drops of 2% $CaCl_2$. What is the result? Why?

9. Dilute 2 c.c. of salted plasma (?) with 10 volumes of water. What happens?

10. Do the same to serum. What is the difference and why?

11. How would you identify in the serum the presence of an albumin and globulin?

Make a list of all the substances which the above reactions have demonstrated.

FIBRIN.

Apply 3 color-proteid tests.

OXYHÆMOGLOBIN.

Place on a glass slide one drop of defibrinated dog's blood. To this add one drop of water and mix with a platinum wire. Allow the mixture to evaporate at room temperature until the edges of the drop have begun to dry. Then place a cover-glass on the slide and examine under the microscope. Sketch the crystals of oxyhæmoglobin.

HÆMIN—TEICHMANN'S CRYSTALS.

Hæmin is the HCl ester of the anhydride of hæmatin.

Place one drop of NaCl solution upon a microscopic slide and allow it to evaporate to dryness. Then add a very small drop of blood and two drops of glacial acetic acid and cover with a glass. Warm *cautiously* until bubbles of gas begin to form in the mixture under the cover-glass. Examine and sketch under the microscope. The hæmin crystals are rhombic plates, brown in color by transmitted light. In large quantities they have a metallic lustre and appear steel-blue by reflected light.

SPECTROSCOPIC EXAMINATION.

(*a*) *Oxyhæmoglobin.*—Dilute 1 c.c. of blood with 100 c.c. of water. Examine spectroscopically. At this dilution one broad absorption band is seen extending from the *D* line (?) (588) to *b* (518). The violet end of the spectrum is also absorbed to *F* (486). Upon diluting this strength solution with an equal volume of water and examining, it is noticed that the broad band has resolved itself into two, the one next to *D* narrower and more intense than the broader one to the right. Between the two bands is a green interspace. Less of the violet end is now absorbed. Upon further dilution, the bands gradually disappear simultaneously.

(*b*) *Hœmoglobin* (*Reduced Hœmoglobin.*)—Prepare some *Stokes' reagent* as follows: Dissolve 3 grams of ferrous sulphate in a small quantity of water and add to it in watery solution 2 grams of tartaric acid. Make up the mixture to 100 c.c and just before using add NH_4OH until the precipitate which at first forms is dissolved. This solution of ammonium ferrotartrate is a reducing agent, removing the oxygen which is in weak combination with the oxyhæmoglobin, forming reduced hæmoglobin. To the 100 times diluted blood add a few drops of Stokes' reagent. Notice change in color. Examine in the spectroscope. A broad less sharply defined absorption band is seen occupying as much space on the spectrum as the two bands of oxyhæmoglobin, but the hæmoglobin band is moved further to the left. If this solution is shaken in the air the color returns to that of oxyhæmoglobin and the latter's characteristic spectrum also reappears.

(*c*) *Methœmoglobin.*—Add to blood (diluted 1:15) two drops of a freshy prepared solution (10%) of potassium ferricyanide. The color of the blood becomes brown. The spectrum, in addition to two bands corresponding nearly to those of oxyhæmoglobin, which, however, are only faintly seen, shows a band in the red near to *C*. If to such a solution, while still in position before the spectroscope, a drop or two of Stokes' reagent is added, the characteristic absorption bands of oxyhæmoglobin appear for a second and are then quickly replaced by those of hæmoglobin. Shaking in the air causes the latter to be reoxidized to oxyhæmoglobin with the consequent spectrum change.

(*d*) *Hæmochromogen, or Reduced Hæmatin.* — To blood (diluted 1:15) add two or three drops of strong NaOH and warm gently until the color changes to a brownish green. Then cool and add two drops of Stokes' reagent. Such a solution shows in the spectrum two very dark bands coinciding apparently [with those of oxyhæmoglobin; upon careful examination, however, it will be seen that green light appears on the left of the left band and consequently both bands are moved further to the right than the oxyhæmoglobin ones.

(*e*) *Hæmatoporphyrin* (*Iron-free Hæmatin*).— Place a few c.c. of concentrated H_2SO_4 in a test-tube and add a drop of blood, mixing well. The color changes to wine-red and spectroscopically the solution shows two bands on the opposite side of the *D* line. The one to the left is narrower and weaker, while that to the right is much more intense, broader, and more sharply defined. This spectrum is very characteristic.

(*f*) *Carbon Monoxide Hæmoglobin.*—Carbon monoxide hæmoglobin may be prepared by passing ordinary illuminating-gas through defibrinated blood until the latter assumes a carmine or cherry-red color characteristic of the combination of CO with hæmoglobin. Examined spectroscopically such a solution shows two bands similar to oxyhæmoglobin except that the bands of the CO-hæmoglobin spectrum are nearer to

the violet end. Add Stokes' reagent to the solution; the spectrum remains unchanged.

Make the following tests, first with diluted blood (oxyhæmoglobin) and then with diluted CO-hæmoglobin; note the differences carefully:

1. Add to 5 c.c. of the blood 3 c.c. $(NH_4)_2S$.
2. " " " " " " 10 c.c. of Stokes' reagent.
3. " " " " " " 10 c.c. of a 20% potassium ferrocyanide and 3 c.c. of acetic acid.
4. Shake with air.

What is characteristic and important in the combination of CO and hæmoglobin as shown by the above experiments? Make drawings of all the spectra seen and compare them with those in the text-books.

MILK.

1. Examine under the microscope fresh milk and skimmed milk. What are the differences?

2. Take the specific gravities of the two and explain the differences.

3. Test the reaction with litmus-paper.

4. Saturate 10 c.c. of milk with $MgSO_4$. What is the precipitate?

5. Shake 5 c.c. of milk with ether. What takes place? Now add a few drops of NaOH. Explain this change.

QUALITATIVE SEPARATION OF THE CONSTITUENTS OF MILK.

Dilute 100 c.c. of milk with 400 c.c. of water and add dilute acetic acid drop by drop until a white flocky precipitate (?) separates out, leaving a fairly clear solution. Allow the precipitate to settle; then filter it off and let it drain as dry as possible. Remove the white precipitated *caseinogen* from the paper and place it in a flask with 100 c.c. of a mixture one half ether and one half alcohol. Allow the precipitate to stand in contact with the ether for an hour, shaking from time to time. Then filter off the ether mixture and allow it to evaporate spontaneously. The residue consists of most of the fat of the milk, mechanically precipitated adherent to the caseinogen. Keep both the caseinogen and the fatty residue.

Place the filtrate from the first acetic acid precipitation in an evaporating-dish and boil over a free flame. The solution being acid, any coagulable proteid not precipitated with the acetic will be rendered insoluble by the heat. This may be filtered off (keep) when the solution is evaporated to about one half its original volume and the clear filtrate again set evaporating until crystals (?) begin to appear, separating out of the solution (now about 20 c.c.).

After allowing the solution to cool, filter off the crystals of calcium phosphate and again place the filtrate over the water-bath and allow it to evaporate to a thick syrup. Of what is this composed?

From the milk have now been isolated—

1. Caseinogen. 2. Fat. 3. Coagulable proteid. 4. Calcium phosphate. 5. Lactose.

CASEINOGEN.

(*a*) Make three color-proteid tests.

(*b*) Test for phosphorus.

(*c*) Dissolve some in very *dilute* NH_4OH and reprecipitate with acetic acid.

FAT.

(*a*) Place in the evaporating-dish which contains the fatty residue 25 c.c. of sodium alcoholate (NaOH dissolved in alco-

hol) and put the dish over the water-bath. Heat the mixture, adding alcohol from time to time to replace that lost by evaporation, until a drop is found to be soluble in water. What is this procedure called? Write the equation. Now evaporate off the alcohol, replacing it by water, and when this is completed add dilute H_2SO_4 until the precipitation is finished. Notice odor. To what is it due? Write this equation.

PROTEID.

Make three color-proteid tests.

CALCIUM PHOSPHATE.

Dissolve some in dilute HNO_3.

(*a*) Add an equal volume of ammonium molybdate and warm to 70° C. What is the precipitate? Filter and dissolve in NH_4OH and reprecipitate as ammonio-magnesium phosphate.

(*b*) Make a portion of the HNO_3 solution alkaline with NH_4OH and reacidify with acetic acid. Add a few c.c. of ammon. oxalate. Examine microscopically.

LACTOSE.

(*a*) Make Fehling's, Moore's, and Nylander's tests.

(*b*) Place 5 c.c. of the lactose solution in a test-tube and add a few drops of Na_2CO_3. Then drop in a little freshly prepared potassium ferricyanide. On heating, a colorless solution results. Is this reaction characteristic of lactose alone? Explain its chemistry.

(*c*) Make fermentation test.

URINE.

PHYSICAL PROPERTIES.

The following comprise the important physical properties of urine to be specially noted in an examination:

Color.—Refer to Vogel's color chart. Relatively unimportant unless the color is other than that of some shade of yellow.

Odor.—Normal, ammoniacal, or that peculiar to some substance not normally present, as acetone, methyl mercaptan, etc.

Transparency.—Clear or cloudy. Usually upon standing, a cloud of mucus-like substance separates, suspending itself in some part of the urine.

Reaction.—Acid, neutral, or alkaline.

Free acid is *never* present, the normal acidity being due to the presence of acid salts such as H_2NaPO_4. HNa_2PO_4 is also present, and by a proper relationship between the dihydrogen and monohydrogen phosphate a neutral or amphoteric reaction may prevail (Litmus). Under normal conditions the 24-hour urine is never alkaline. Portions of such urine drawn a few hours after digestion may react alkaline, due to the withdrawal of acidic radicals from the blood in the formation of the acid gastric juice. Alkaline urine may be caused by free or fixed alkalies. When this is the case the urine is always cloudy or turbid. Care should be taken that the observed alkalinity is not due to decomposition after drawing. Volatile alkalies such as ammonia react blue to litmus, but upon warming the paper the red color returns. This will not occur if the reaction is caused by a fixed alkali. Organic acids are burnt in the body to carbonates and the latter

decrease the acidity, sometimes, in fact, causing an alkaline reaction to appear.

Specific Gravity.—Varies considerably from 1015-1025. It is dependent upon the amount of water ingested and that excreted by the bowels, skin, and breath.

Determination is made by means of the urinometer (hydrometer). If there is a sediment the urine must be warmed, filtered, and allowed to cool.

Place the cylinder upon the table and fill it to within an inch of the top with urine; then immerse the urinometer. Read off on the graduation of the spindle the place where the meniscus of the fluid cuts it.

Sediments.—Notice should be taken as to their color, amount, character, etc. The method for the determination of the identity of such will be taken up later.

Total Solids.—These may be calculated approximately by multiplying the second and third decimals of the specific gravity by Häser coefficient $=2.33$. This gives the number of grams in 1000 c.c. of the urine, from which must be calculated the total amount in the 24 hours.

TESTS FOR NORMAL CONSTITUENTS OF URINE.

Inorganic Acidic Radicals.

(*a*) *Chlorides.*—These are present combined principally with Na and K. Acidify some of the urine with HNO_3 and add a drop of $AgNO_3$. Prove that this precipitate is AgCl.

(*b*) *Sulphates.*—These occur in two forms, viz., preformed or sulphate sulphuric acid and ethereal or combined sulphuric acid. The former precipitates directly from the urine by the addition of $BaCl_2$ in an acid reaction, the latter only after previously boiling the urine with a mineral acid. Acidify some urine with acetic acid and add a few drops of $BaCl_2$. Prove that this precipitate is $BaSO_4$. Filter (filtrate must be clear). To this add a few c.c. of concentrated HCl and boil a few minutes. Then add a few c.c. of $BaCl_2$. A turbidity is indicative of the presence of ethereal sulphates.

Sulphur in the unoxidized, organic, or neutral form may also be present in the urine as a constituent of taurin, cystin, KSCN, etc. Place some urine in a flask with a few pieces of pure zinc and add enough HCl to cause a gentle evolution of gas. Partially close the mouth with a piece of filter-paper moistened with lead acetate. In the presence of organic sulphur the paper is blackened. Explain this.

(*c*) *Phosphates.*—These are present combined with NH_4, Na, K, Ca, and Mg as primary, secondary, or tertiary compounds.

1. Make some urine alkaline with NH_4OH. What is the precipitate? Filter and to the clear filtrate add two drops of $BaCl_2$. What is this new precipitate? Filter this off through the same filter as the first and dissolve the whole precipitate in dilute HNO_3. Test for phosphates.

2. Make the urine acid with acetic acid and add a few drops of uranium nitrate solution. Of what is the precipitate composed?

3. Acidify the urine with a few c.c. of HNO_3 and add some molybdic solution. Warm at 80° C. What is this precipitate?

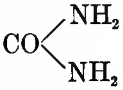
4. Boil about 10 c.c. of the urine with one quarter its volume of Fehling's solution. Notice the color of the precipitate. To what is it due? Then boil 10 c.c. of Fehling's solution with one quarter its volume of urine. What occurs? Contrast these two experiments. Write all the equations for the reactions in the above experiments.

Basic Radicals.

With the exception of NH_4 these have little or no chemical significance. The proof for the presence of ammoniacal compounds will be taken up under the quantitative determinations.

Organic Constituents.

Urea.



$$CO\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix}$$

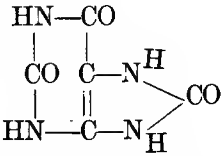
Make use of the synthetic urea crystals for the following reactions:

(*a*) Dissolve some urea in a few c.c. of water. Place in each of two watch-glasses one drop of the solution. To one add one drop of HNO_3 and to the other a drop of concentrated oxalic acid solution. Allow them to stand and then examine the crystals under the microscope. What are these compounds?

(*b*) Warm carefully a few crystals of urea in a dry test-tube. The substance melts, giving off NH_3. Continue heating until the fused mass solidifies. Cool the test-tube and add a few c.c. of water and NaOH drop by drop until the residue is all in solution. Add now a drop or two of very dilute $CuSO_4$ solution. To what reaction does this correspond? What substance is formed from the urea? Write the equation.

(*c*) To 5 c.c. of NaOH add some bromine water and drop in a crystal of urea. What is the reaction which takes place?

Uric acid.



Make use of the crystals which have been collected from acidifying the normal urine.

1. Examine crystals under the microscope. Sketch various crystals.
2. Test solubility in water, NaOH and NH_4OH.

3. Dissolve some of the crystals in a few c.c. of dilute NaOH and add NH_4Cl to saturation. What is the precipitate?

4. Heat a crystal on platinum foil.

5. Dissolve some uric acid in a small quantity of dilute NaOH. Add concentrated H_2SO_4 carefully drop by drop until the solution is too warm to touch; then add potassium permanganate. What is the reaction which takes place?

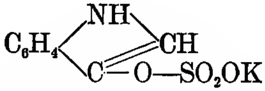
6. Make a concentrated solution of uric acid and pour it into 10 c.c. Fehling's solution which has previously been brought to boiling. Observe and note result.

7. Dissolve some uric acid in dilute Na_2CO_3 and place a couple of drops on some filter-paper previously moistened with $AgNO_3$. To what is the blackening due?

8. *Murexide Test.*

Place a couple of crystals in a clean dry evaporating-dish and pour upon them two drops of concentrated HNO_3. Evaporate to dryness *very carefully* over a free flame. A yellowish residue results which upon cooling and the addition of a drop of NH_4OH becomes purple-red. If NaOH instead be used the color will be purple-violet.

Indican = potassium indoxyl sulphate.



The following tests are based upon the oxidation of the indican to indigo.

1. *Jaffe's Test.*—To about 10 c.c. of urine add 2 or 3 c.c. of chloroform and mix well with an equal volume of concentrated HCl. Then add drop by drop, shaking well between each drop, a concentrated solution of chloride of lime. The indigo (?) is dissolved by the chloroform.

2. *Obermayer's Test.*—Perform the test in the same manner except instead of concentrated HCl and lime add an equal volume of Obermayer's reagent (conc. $HCl + Fe_2Cl_6$).

3. *Hammarsten's Test.*—The same test, using Hammarsten's reagent (?). Compare results.

UROBILIN.

1. To 5 c.c. of urine add 3 drops of basic lead acetate. Shake thoroughly. Of what is the voluminous precipitate composed? Filter; notice that the filtrate is colorless and clear. In what way is the coloring matter of the urine precipitated?

2. To 50 c.c. of urine faintly acidified with H_2SO_4 add ammonium sulphate (in substance) to saturation. Filter off the precipitate and dissolve it in alcohol containing a few drops of concentrated HCl. What sort of a body is urobilin?

3. To 50 c.c. of urine add an equal mixture of neutral and basic lead acetate until the precipitation is complete. Filter and allow the precipitate to drain as dry as possible. Then place it and the filter-paper in an evaporating-dish half full of 95% alcohol acidulated with HCl. Warm over the water-bath until the alcohol is well colored. Filter and examine the solution in the spectroscope. Evaporate off the alcohol and replace it by water to which a few drops of NH_4OH has been added; now add some zinc chloride solution. Note the fluorescence.

PHENOL.

Treat the urine with $\frac{1}{5}$ its volume of dilute H_2SO_4 and distil off $\frac{1}{1}$ of the volume of the solution. Use this distillate for the following reactions:

1. Add some Millon's reagent to a little of the solution. Warm. Compare this with test under proteid and tyrosin.

2. To a few c.c. of the solution add a trace of *neutral* ferric chloride solution. When did you use this reaction before? Add a drop of HCl.

3. To 5 c.c. of the solution add a few c.c. of bromine water. What is this yellow precipitate?

Oxalic Acid.

To 500 c.c. of urine add 5 c.c. of $CaCl_2$ solution. Then make slightly alkaline with NH_4OH and finally acid with acetic acid. After standing 24 hours collect the precipitate (?) upon a small filter. Treat the precipitate with 10 c.c. of very dilute HCl. Some of the precipitate dissolves. What remains? Filter and to the filtrate made again first alkaline with NH_4OH and then acid with acetic acid, add $CaCl_2$ (few c.c.) and allow to stand. Examine the crystals under the microscope.

QUANTITATIVE DETERMINATIONS.

Chlorides.

The principle of the method is the following:

To the urine is added an excess of $AgNO_3$ over and above what is necessary to precipitate all the chlorides present. The excess of Ag is then determined by means of a sulphocyanide solution, using iron alum as an indicator.

Reagents necessary:

1. A $AgNO_3$ solution, each c.c. of which precipitates 0.01 grm. NaCl (29.075 grms. $AgNO_3$ in a liter).
2. A saturated solution of iron alum.
3. Chlorine-free HNO_3 of a specific gravity 1.2.
4. A potassium sulphocyanide solution of which 2 c.c. corresponds to 1 c.c. of the known $AgNO_3$ solution.

Method: Prepare a *clean* and *dry* graduate. By means of a pipette measure off *accurately* 5 c.c. of urine and run it into the graduate. Add 3 c.c. of the HNO_3 and dilute with water to about 25 c.c. Then allow exactly 10 c.c. of the known

$AgNO_3$ solution to flow in. Mix the solution well and fill up with water to 50 c.c. on the graduate. Transfer this now to a clean and dry beaker and clean and dry the graduate again. Prepare also a clean and dry funnel with paper and filter the mixture in the beaker into the graduate. When 25 c.c. of a water-clear filtrate has passed through remove the funnel to a test-tube. Add 10 drops of the iron alum to the graduate and titrate with the known potassium sulphocyanide until the first tinge of red appears in the solution.

Calculate the amount of chlorine or NaCl in the 5 c.c. of the urine used.

SULPHATES.

The sum of the preformed and ethereal sulphates makes up the total sulphate. The procedure for the determination of these various units rests upon the following facts:

Boiling the urine with dilute HCl liberates all the sulphate radicals present, in such a form that they may be precipitated with $BaCl_2$ as $BaSO_4$. This gives the amount of total sulphate. If acetic acid be used instead of HCl, the resulting precipitate with $BaCl_2$ will be made up of the preformed or sulphate-sulphate. This may be filtered off and the filtrate treated as for the total sulphates, the result being the amount of ethereal sulphates present. The difference between this and the total will be the amount of the preformed sulphate.

TOTAL SULPHATES.

In a clean beaker place 50 c.c. of urine and dilute it with 100 c.c. of water, adding 5 c.c. of HCl. Heat just to boiling and after adding 10 c.c. of the $BaCl_2$ solution allow to cool and stand *covered* in a cool place for 24 hours. Filter through a small ash-free filter; the precipitate must be removed *quantitatively* from the beaker to the paper by means of warm water. Now wash the white precipitate with water until the washings give no test for chlorine; then dry at 100° C. When dry, slip out the paper from the funnel, fold it up so that the contained sulphate cannot fall out and place it in a porcelain crucible which has previously been ignited and weighed. Ignite the paper in the crucible carefully and burn until the residue (?) is white. Cool the crucible and weigh. The difference in the two weights will give that of the $BaSO_4$, from which may be calculated the SO_4 in the total day's urine.

PHOSPHATES.

The sum of the alkaline and earthy phosphates equals the total phosphate.

TOTAL PHOSPHATE.

The procedure is based upon the quantitative precipitation of *all* the phosphates in the urine with uranium nitrate, as uranium phosphate, using potassium ferrocyanide as indicator. The phosphates must be present as acid salts.

Reagents necessary:

1. A uranium nitrate solution 1 c.c. of which equals 0.005 P_2O_5 (35.461 grms. in a liter).

2. An accessory solution (100 grms. of sodium acetate and 300 grms. of acetic acid in a liter).

3. A solution of potassium ferrocyanide. What is the reaction which indicates the absolute precipitation of all the P_2O_5?

Method : Place 25 c.c. of urine in a large evaporating-dish, add 5 c.c. of the accessory solution (why?) and warm gently over an asbestos board. Keeping the solution warm, add the known uranium nitrate solution from a burette, from time to time removing a drop of the urine on the end of a glass rod and adding it to a drop of the potassium ferrocyanide which has been placed upon a white porcelain dish. When all the

P_2O_5 has been precipitated by the uranium and the first excess of the latter appears in the urine solution, the drop of indicator on the plate when tested as above will take on a *permanent faint* reddish-brown color. It is important that the color be permanent. If the color disappears upon standing, more uranium must be added and retested.

Calculate the total amount of P_2O_5 in the 24-hour urine.

EARTHY PHOSPHATES.

Place 50 c.c. of urine in a beaker and make it alkaline with NH_4OH. A precipitation of the earthy phosphates occurs. Allow this to stand for a couple of hours and then collect the precipitate upon a small filter. After having washed the precipitate with very dilute NH_4OH transfer it quantitatively to an evaporating-dish by means of acetic acid (1:2), which dissolves the P_2O_5. Dilute the solution to about 25 c.c. and titrate as outlined under total phosphates. The difference between this amount and that of the total will be the amount of alkaline P_2O_5.

Phosphorus exists in the urine also in organic combination probably as glycero-phosphoric acid. This form is not precipitated by uranium nitrate. Determination is made by evaporating the urine to dryness, fusion of the residue with fusion mixture, and final solution of the resulting mixture in water made acid with acetic acid. The titration of such a solution with uranium nitrate will give the amount of *total phosphorus* in the urine.

DETERMINATION OF THE ACIDITY OF THE URINE.

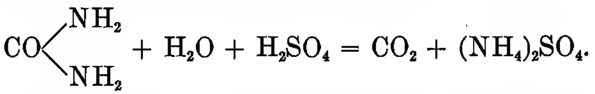
The degree of acidity of a urine is dependent upon the ratio between the mono- and dihydrogen phosphates present. The monohydrogen (HM_2PO_4) phosphates are precipitated by $BaCl_2$, while the dihydrogen (H_2MPO_4) phosphates, in the dilution in which they are present in the urine, remain in solution. If the former, therefore, be removed by precipitation with $BaCl_2$ and filtration, and the P_2O_5 in the filtrate be determined by titration with uranium nitrate, the difference between this figure and the amount of total phosphate will determine the amount of the monohydrogen phosphate which was precipitated with $BaCl_2$. A ratio can thus be established between the two.

Method : Treat 75 c.c. of urine with 15 c.c. of $BaCl_2$ and mix thoroughly in a beaker. Filter through a dry filter until 60 c.c. of a *clear* filtrate is obtained. Determine the P_2O_5 in this as under total phosphate. Then determine the ratio between the mono- and dihydrogen phosphates. Make 3% correction (?).

Total Nitrogen (Kjeldahl).

This method embraces the determination of nitrogen combined with hydrogen, as $N=H$, NH_2, and NH_3.

The principle of the method consists in the decomposition of the nitrogenous bodies by means of H_2SO_4, during which process the carbon is oxidized to CO_2 and the hydrogen to H_2O. The nitrogen is converted to NH_3, which combines with the SO_4 radicle, forming $(NH_4)_2SO_4$. For example, urea decomposes according to the following reaction:



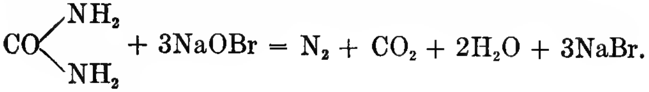
$$CO\begin{matrix} NH_2 \\ NH_2 \end{matrix} + H_2O + H_2SO_4 = CO_2 + (NH_4)_2SO_4.$$

To this acid solution containing $(NH_4)_2SO_4$ enough alkali is then added to combine with all the SO_4 present. This liberates the NH_3, which is distilled over into a known amount of acid.

For the details of the method see demonstration.

UREA (HÜFNER'S METHOD).

The method is based upon the following reaction:



$$CO\begin{matrix}NH_2\\NH_2\end{matrix} + 3NaOBr = N_2 + CO_2 + 2H_2O + 3NaBr.$$

See Experiment (*c*) under Urea.

Method : The urine must be of such a dilution that it does not contain more than 0.5% urea. This is easily accomplished when the specific gravity is known.

Rinse the ureometer first with water and then fill it with the hypobromite solution, so that when the apparatus is perpendicular and no air is at the top, the amount of fluid in the bulb covers the opening from it to the upright tube. Then suck up with the pipette the exact amount of urine, and placing it under the surface of the solution with the pipette tip well into the space below the upright tube, force the urine out of the pipette slowly, noting that the bubbles (?) generated all pass upward into the closed tube. Care must be taken that the last drop of urine is expelled from the pipette without allowing any air to escape from it also. Allow the reaction and collection of gas to go on for half an hour, then make a reading on the scale on the areometer.

PROTEID.

Fill the albuminometer to the mark "*u*" with acidified urine and add Esbach's reagent to "*R*" (reagent = 10 grms. picric acid + 20 grms. citric acid dissolved in a liter of water). Allow the precipitate to stand 24 hours and then read the height of the precipitate on the scale, which gives the number of grms. of proteid in a liter.

URIC ACID (HOPKINS' METHOD).

The principle of the method rests upon the precipitation of uric acid by ammonium chloride as ammonium urate.

Method: Place 100 c.c. of urine in a beaker and add 30 grms. of NH_4Cl. Stir until all the salt is dissolved and then allow to stand 2 hours. The precipitate is then collected upon a small filter-paper and washed with a saturated solution of NH_4Cl. Dissolve the precipitate on the paper by pouring on it 50 c.c. of water acidulated with HCl and allow it to flow into a small beaker. Place the beaker on the water-bath and evaporate the solution to about 5 c.c. Allow to stand for one hour. Filter off the crystalline precipitate on a small filter and after washing once with acidulated water make a nitrogen determination on the filter-paper and precipitate according to Kjeldahl.

From the result may be calculated the amount of uric acid

AMMONIA (SCHLÖSING METHOD).

The method is based upon the liberation of NH_3 from its salts by the use of a weak base such as milk of lime at room temperature and the catching of the NH_3 so set free in a closed space by a known amount of acid.

Method: Set upon the base of an exsiccator a round flat-bottomed dish in which has been placed 25 c.c. of *fresh, filtered* urine. Lay upon the edges of the dish a glass triangle and rest upon the latter another smaller circular dish containing 20 c.c. of $\frac{n}{4}H_2SO_4$. Then add 10 c.c. of milk of lime to the urine and place the cover over the exsiccator quickly. Allow the apparatus to stand 5 days, after which time titrate the acid with $\frac{n}{4}NH_4OH$. The difference between the number

of c.c. of the alkali necessary to neutralize the $\frac{n}{4}$ acid and 20 corresponds to the amount of NH_3 liberated from the 25 c.c. of urine.

SUGAR (POLARIZATION).

See demonstration.

PATHOLOGICAL URINARY CONSTITUENTS.

ALBUMIN AND GLOBULIN.

In testing urines suspected of containing proteid, the fluid should always be perfectly clear. This can be accomplished either by repeated filtration through paper or asbestos or by shaking with magnesia.

The following tests are best suited for ordinary conditions:

1. *Heat Test.*—Warm 5 c.c. of clear urine to boiling and add 3 to 6 drops of dilute acetic acid. If the urine contains more than a small amount of albumin, this will settle out in flocks after the addition of acid. When mere traces are present the solution may only become turbid and should then be compared to the urine before heating, but to which the same amount of acid has been added. This test is only of value as a *positive* one. When a negative result is obtained other tests should be tried with a view to confirmation.

In some cases the addition of acetic acid to boiled urine may give rise to a faint precipitate or turbidity which disappears upon shaking. This is caused by the formation of acid albumin, which may be salted out with $\frac{1}{3}$ volume of saturated NaCl after the addition of more acetic acid to keep the phosphate in solution. Faintly alkaline or amphoteric urine may sometimes give on heating a precipitate due to phosphates, which is sometimes difficult to distinguish from a precipitate of albumin. Again, such a urine may remain perfectly clear and still contain albumin. The addition of a small amount of acid will, in the first case, dissolve the phosphate and in the second precipitate any albumin remaining soluble. Nucleoalbumins nor albumoses do not respond to this test, as both bodies are soluble in hot acid solutions. A precipitate settling out upon cooling may point to the presence of such substances. Resins resulting from the administrations of petroleum, turpentine, oil of sandal-wood, tolu-balsam, etc., may be present in the urine, and if so will be precipitated by the acid. Such precipitates easily dissolve in alcohol.

2. *Heller's Test.*—Stratify in a test-tube 5 c.c. of HNO_3 and 5 c.c. of urine. If albumin be present in the urine, in conjunction with the HNO_3 at the surface of contact of the two liquids it will be precipitated and will appear as a *white ring where the liquids meet.* As the HNO_3 upon standing diffuses upwards into the urine the ring may become broader. This test is sensitive enough for ordinary clinical purposes, but will not show the presence of traces. Urines containing an excessive amount of urea may form a crystalline precipitate (?) in this test, but such a precipitate cannot be confused with albumin. Colored rings may also form, due to the oxidation of urinary pigments, indican producing a blue coloration (?). Substances mentioned under the heat test, such as resins, etc., may form a cloud, but simple tests such as those indicated will exclude them.

3. *Roberts' modification of Heller's Test.*—Instead of HNO_3 this reaction makes use of a reagent (1 part concentrated HNO_3+5 parts saturated $MgSO_4$ solution) which is stratified in the same way as in Heller's test. It has some optical advantages and colored rings never appear in its use.

4. *Acetic acid and potassium ferrocyanide Test.*—Acidify 5 c.c. of urine with two drops of acetic acid and add drop by drop a dilute solution of K_4FeCN_6. In the presence of albumin a white precipitate occurs which is soluble in an excess of the reagent. Traces of albumin may be detected with this reaction. Should the precipitate dissolve upon heating, albumoses may be suspected. The presence of a considerable amount of mucin or nucleoalbumin in the urine may sometimes give rise to a precipitate with acetic acid alone. This must be removed by filtration before the ferrocyanide test can be completed.

5. *Trichloracetic acid Test.*—Stratify a few c.c. of a concentrated aqueous solution of this reagent with 5 c.c. of urine. A white ring sharply defined indicates the presence of albumin. The precipitate may also be albumoses, but in this case the ring dissolves with cautious warming. This test is more sensitive than Heller's, and by its use smaller quantities of albumin are demonstrable in urines with which the more common tests yield negative results.

6. *Spiegler's Test.*—Stratify 5 c.c. of urine slightly acidified with acetic acid with a few c.c. of Spiegler's reagent (8 grms. $HgCl_2$, 4 grms. tartaric acid, 20 grms. glycerin in 200 c.c. of water). In the presence of albumin a white ring appears at the line of contact of the two liquids. This test is very sensitive, showing albumin in a dilution 1:250,000. In fact most normal urines indicate albumin with the reagent. This must be borne in mind in making deductions from its use. Urines containing iodides give a precipitate of HgI_2.

7. *Tanret's* (*Bouchardat*) *Test.*—The reagent ($HgCl_2$ and KI dissolved in water and to this glacial acetic acid added) is added, drop by drop, to the urine until a turbidity or precipitate appears. The reagent precipitates, besides albumin, mucin, peptone, and alkaloids. In cases where the presence

of alkaloids in the urine is suspected, the peptone and alkaloids may be dissolved in potassio-mercuric iodide and the solution shaken out with ether, whereby the alkaloid is dissolved.

PROTEOSES.

In general, as pointed out in the previous tests, the proteoses may be differentiated from the albumins and globulins by the greater solubility of their precipitates by heat or in an excess of the reagents.

PEPTONE.

Urines containing peptone show no test upon heating, are not precipitated with mineral or acetic acids, nor do they give the acetic acid and potassium ferrocyanide reaction. Treated with acetic acid and phosphotungstic acid such urines give a precipitate upon standing.

DEXTROSE.

Before testing for dextrose in the urine, proteid, if present, must be removed by heat and acetic acid. The following tests depend upon the power of dextrose to reduce metallic oxides as evidenced by the formation of precipitates or color changes. It must be remembered that the urine may also contain other bodies such as creatinin, uric acid, allantoin, hydrochinon, alkaptonic acid, urine and bile pigments, and conjugate glycuronic acids, which also reduce, in a slighter degree however, metallic oxides. It is, therefore, better never to base a decision entirely upon reduction tests.

Never allow the solution to boil more than a few seconds in performing the tests. This will tend to eliminate the possibility of a reduction due to the above-mentioned substances.

1. *Trommer's Test.*—Perform this test as indicated under Dextrose, using urine instead of the dextrose solution.

2. *Fehling's Test.*—Perform this as under Dextrose, having in mind Experiment 4, under Phosphates.

3. *Boettger's Test.*—Make 5 c.c. of the urine alkaline with NaOH and add a little powdered bismuth nitrate. Warm the solution gently for a few moments. The dextrose reduces the nitrate to oxide or even to metallic bismuth, as shown by the blackening of the white precipitate. This test has the advantage that Bi is harder to reduce than Cu or Ag, and therefore many reducing bodies in the urine do not affect it.

4. *Nylander's Test.*—To 10 volumes of urine add 1 volume of Nylander's reagent and boil. The presence of sugar is indicated by a dark coloration of the urine followed by a separation of a black precipitate (?). By this very sensitive test the reducing character of some normal urines may be shown. Sulphide containing urines cannot be tested by this method. Why?

5. *Phenylhydrazin Test.*—Perform as given under Dextrose, using 15 c.c. of urine. As other substances in the urine may give a precipitate with the reagent, a mere separation of an insoluble body is not sufficient evidence for the presence of a sugar. The precipitate must be yellow and must be examined *carefully* and *critically* under the microscope. If sufficient quantities are obtainable for a melting-point determination, this procedure should be carried out. Phenylglucosazone melts at 204–205° C.

6. *Fermentation Test.*—Perform as suggested under Dextrose.

7. *Polarization Test.*—The sensitiveness of this test is dependent entirely upon the instrument employed, the average of which will not indicate polarization when reduction tests are negative. The urine must be proteid-free and most of

the coloring matter must have been removed by shaking with lead acetate in substance and filtration.

BILE.

Notice the color of the urine, but do not confound it with that voided after the ingestion of rhubarb, santonin, etc.

In order to insure a positive identification of icteric urine by means of the bile *acids* it is necessary to separate them from the urine by a long and laborious procedure and then perform Pettenkofer's test. Though this is the more sensitive method, still it is more usual clinically to perform tests for the biliary *pigments* directly on the urine.

1. Perform Gmelin's, Smith's, Hammarsten's, and Huppert's tests.

2. *Rosenbach's modification of Gmelin's Test.*—Filter some icteric urine and to the moistened paper add *one* drop of HNO_3. Colored rings around the drop correspond in color and arrangement to those obtained in Gmelin's test. Caution: Impure filter-paper may give this test, so make a control test in using strange paper.

3. *Hay's Test.*—Fill a test-tube half full of urine and sprinkle on the surface powdered sulphur. If the sulphur sinks to the bottom the presence of bile salts is indicated.

BLOOD.

The presence of blood in urine is usually proven by adducing evidence as to the presence of blood-coloring matters —hæmatin or its proteid compounds. The urine may contain corpuscles, but these must be sought for in the sediment. The following bodies may be present: Oxyhæmoglobin, methæmoglobin, and hæmatin. Reduced hæmoglobin is never present in the urine.

OXYHÆMOGLOBIN.

1. Notice the color of the urine. If fresh it has a reddish tinge and is turbid.

2. Examine with spectroscope. If the urine is not fresh be on the lookout for methæmoglobin. Treat a portion of the urine with an excess of NaOH which contains some sodium sulphide. Oxyhæmoglobin is changed to hæmochromogen with its characteristic spectrum.

3. *Heller's Test.*—Make some urine strongly alkaline with NaOH and boil it. The oxyhæmoglobin is split into hæmatin and proteid, and the earthy phosphates being precipitated, drag down the hæmatin with them. The precipitate should be red. Filter off and dry the precipitate and test it for Teichmann's crystals.

4. *Struve's Test.*—Make a portion of urine alkaline with NaOH and precipitate with tannic acid. Test this precipitate for hæmin.

METHÆMOGLOBIN.

Examine with the spectroscope. Add $(NH_4)_2S$ as suggested under Blood and get the spectrum of reduced hæmoglobin. Do not confound the spectrum of methæmoglobin with that of hæmatin in acid solution.

HÆMATIN.

1. Examine in the spectroscope. If a single band is present add $(NH_4)_2S$ to the urine, filter, and again examine. The two bands of reduced hæmatin should appear.

2. Make tests 3 and 4 under Oxyhæmoglobin.

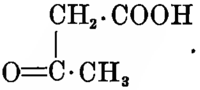
HÆMATOPORPHYRIN.

The use of sulphonal and similar medicinal agents give rises to changes in the blood which show themselves by the presence of hæmatoporphyrin in the urine.

1. Examine with the spectroscope. The presence of other coloring matters renders such observations unsatisfactory and misleading.

2. *Method of Garrod.*—To 100 c.c. of urine add 20c. c. of 10% NaOH. The phosphates of the earthy metals are precipitated and with them the hæmatoporphyrin. This precipitate is filtered off, washed, and warmed in a flask with acidulated alcohol. The pigment goes into solutionn. Use this for the spectroscope.

DIACETIC ACID.

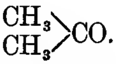


$$\begin{array}{l} \quad CH_2\cdot COOH \\ \quad | \\ O{=}C\cdot CH_3 \end{array}.$$

Urine must be tested soon after voiding, as this body disappears upon standing.

Strongly acidify some urine with H_2SO_4 and shake out with ether. Separate the ether from the solution and shake out the former with water which is just colored with Fe_2Cl_6. The watery solution becomes violet and upon the addition of more Fe_2Cl_6 turns bordeaux-red.

ACETONE.



$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CO.$$

Distil 100 c.c. of urine to which has been added 2 c.c. of 50% acetic acid. Take the first 50 c.c. of the distillate, add 1 c.c. of 8 times diluted concentrated H_2SO_4 and redistil over 25 c.c. Make the following test with this solution.

1. *Lieben's Test.*—Place some of the solution in a test-tube and make alkaline with sodium carbonate. Add enough of

Tanret's reagent to give the solution a decided yellow color. Warm at 65° C. for 5 minutes and allow to cool. A yellow precipitate of iodoform settles out, recognizable by its odor and its hexagonal crystals. This test is also given by alcohol.

2. *Reynold's Test.*—Precipitate a solution of $HgCl_2$ with alcoholic NaOH and add to this a portion of the fluid distillate. Acetone has the power of dissolving freshly precipitated HgO, and the latter's presence may be tested for by filtering the above mixture and adding to the filtrate $(NH_4)_2S$.

3. *Legal's Test.*—To a few c.c. of the distillate add a few drops of a freshly prepared solution of sodium nitroprusside and make the solution alkaline with NaOH. A ruby-red color is produced which quickly disappears. Creatinin also gives this reaction. If in this case the alkaline solution is treated with a large excess of acetic acid the color becomes red, whereas with creatinin it is changed to green and then blue.

EHRLICH'S DIAZO REACTION.

Diazobenzolsulphonic acid comes into prominence as a reagent in various connections.

If a dilute solution of this reagent be added to certain pathological urines (typhoid, pulmonary tuberculosis, etc.), and the mixture then made alkaline with ammonia, the solution becomes carmine or scarlet. Upon shaking, the foam also partakes of a reddish color. Normal urines give a yellowish-red coloration with this reagent. This substance is also used in testing for sugar, proteid, and bilirubin in urine, and the possibilities of error in interpretation may always be borne in mind on this account.

SEDIMENTS.

UNORGANIZED.

Separating from a urine which is *acid* in reaction, the following substances may be present:

1. *Crystalline Type.*

(*a*) *Uric Acid.*—Color, golden brown. To what is this due? Does not dissolve upon warming. Soluble in NaOH and reprecipitated by HCl. Responds to the murexide test. Very characteristic crystalline form under the microscope.

(*b*) *Calcium Oxalate.*—Usually present mixed with uric acid. Colorless. Dissolves easily in HCl, but is insoluble in acetic acid. (See Triple Phosphate, with which there is the possibility of confusion.) Under the microscope the crystals are transparent, refractive, octahedral (envelope shape).

(*c*) *Bilirubin and Hœmatoidin.*—The former crystallizes in golden or brown rhombic plates or needles. Dissolves easily in alkalies and chloroform and gives Gmelin's reaction. The latter is similar in crystalline form, but does not dissolve in alkali and gives a blue coloration with HNO_3.

(*d*) *Cystin.*—Under the microscope it appears as superimposed six-sided plates, which are insoluble in acetic acid, but soluble in NH_4OH (differing from uric acid).

(*e*) *Tyrosin, Leucin, and Xanthin.*—For tests, see under these substances.

(*f*) *Phosphates.*—1. Magnesium Phosphate. Rhombic plates, soluble in acetic acid, slightly attacked by ammonium carbonate. 2. Calcium Phosphate. Soluble in acetic acid. Crystals wedge-shaped, seldom found. 3. Ammonio-magnesium Phosphate (Triple Phosphate). These separate only when the reaction is *weakly acid* or amphoteric.

(*g*) *Potassium Sulphate.*—Long colorless needles, insoluble in NH_4OH or acids; seldom found.

2. *Amorphous Type.*

(*a*) *Uric Acid Salts* (Acid Urates).—Brick-red or brownish-red in color. Dissolves upon warming and gives the murexide test. Upon the addition of a mineral acid, free uric acid separates out in small crystalline form.

(*b*) *Calcium Oxalate.*—Dumb-bell shape. See above for detection.

(*c*) *Calcium Sulphate.*—Dumb-bell shape, insoluble in HCl.

(*d*) *Fat.*—Strongly refracting round drops, soluble in ether.

Separating from an *alkaline* reacting urine:

1. *Crystalline Type.*

(*a*) *Triple Phosphate.*—Dissolves easily in acetic acid; unchanged by ammonium carbonate (see Mag. Phosphate); appears under the microscope as large colorless prisms (coffin-cover shape). Upon warming gives off NH_3.

(*b*) *Ammonium Urate.*—Dissolves in HCl or acetic acid, followed by the separation of free uric acid crystals (rhombic form). Forms dark balls with needles radiating from the circumference (chestnut-burs). Gives off NH_3 upon heating on a platinum foil.

(*c*) *Magnesium Phosphate.*—See under Acid Urine.

2. *Amorphous Type.*

(*a*) *Earthy Phosphate.*—Dissolves in acetic acid without the development of gas.

(*b*) *Earthy Carbonate.*—Dissolves in acetic acid with effervescence.

(*c*) *Calcium Carbonate.*—Dumb-bell shape. Soluble in acetic acid, with an escape of gas. (Compare Calcium Oxalate.)

SCHEME FOR IDENTIFYING SEDIMENTS.

<table>
<tr><td colspan="6">On heating the sediment on a platinum foil it</td></tr>
<tr><td colspan="4">Does not char.</td><td colspan="2">Does char.</td></tr>
<tr><td colspan="4">Fresh sediment treated with HCl</td><td colspan="2">Fresh sediment gives the murexide test.</td></tr>
<tr><td colspan="3">Does not effervesce.</td><td rowspan="5">Effervesces.</td><td colspan="2">Sediment treated with NaOH gives</td></tr>
<tr><td colspan="3">Fresh sediment gently heated and then treated with HCl</td><td rowspan="4">Ammonia.</td><td rowspan="4">No ammonia.</td></tr>
<tr><td colspan="2">Does not effervesce.</td><td rowspan="3">Effervesces.</td></tr>
<tr><td colspan="2">Fresh sediment moistened with NaOH</td></tr>
<tr><td>NH_3</td><td>No NH_3</td></tr>
<tr><td>Triple phosphate.</td><td>Ca or Mg phosphate.</td><td>Calcium oxalate.</td><td>Calcium carbonate.</td><td>Ammonium urate.</td><td>Uric acid or urates.</td></tr>
</table>

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